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# Spectrophotometric determination of Fe (II) in pharmaceutical preparations using heterodiazophenoles

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3-mercapto-5-(2',4'-dihydroxyphenyloazo-1')-1,2,4-triazol (METRIAP) and 5-(5'-mercapto-1,3,4-tiadiazolo-2'-azo)-2,4-dihydroxybenzoic acid (METIDAREZ– $\beta$ ) were used for determination of milligram quantities of Fe (II) in pharmaceutical preparations as Ferrum, Ferrum 4mg +Vit. C, Pan Vitan for women and Tardyferon<sup>R</sup>. The stability of the complexes with Fe (II) was expressed as log K at the pH of 10.3 and with METRIAP it was 16.46, while with METIDAREZ– $\beta$  at the pH of 7.4 it was 14.25. Iron ions (II) after mineralisation and reduction of Fe III to Fe II were determined spectrophotometrically.

Keywords: 2-mercapto-1,2,4-triazole and 2-mercapto-1,3,4-thiadiazole azo dyes, iron determination, spectrophotometry, AAS, pharmaceutical preparations

## 1. INTRODUCTION

The basis for maintaining a healthy organism is the right diet, supplying an individual with energy, proteins, carbohydrates, fats, microelements as provitamins, vitamins and mineral salts (macro and micro-elements). The right amount of vitamins and mineral salts in the diet allows using other food components in the process of matter conversion in building the tissues of the organism and providing them with vital energy. Mineral substances have different functions in the organism; they constitute the building material of bone structures, soft cells and tissues; they are the constituents of enzymes, enzyme activators, hormones, vitamins and system fluids.

One can observe an increase of multivitamine and microelement application in cases of physical and psychical strain, convalescence, anemia, insufficiency of trace elements or vitamins. However, it should be noticed that both insufficiency and the surplus of these substances may lead to serious health problems, which may even sometimes result in death. Common application of preparations containing microelements requires fast and accurate methods of ions determination, especially those of the heavy metals. The most common element in multivitamin preparations with microelements is iron (II). In pharmaceutical preparations, foods, biological material and herbs, this element is determined spectrophotometrically using: or cyprenaline [1], HDEHP (bis-(2--ethylhexylo)-phosphoric acid [2], 3-formylo-4-hydroxy-5-metoxybenzaldehyde [3], 1-(p-talilo)-3-phenylopropano-1, 3-dion [4], Schiff's bases [5], AAS method [6,7,8], fluid injection FIA analysis [9,10,11], capillary electrophoresis [12], ion chromatography [13], HPLC [14,15], electrochemical methods [16,17]. The purpose of this paper was to examine the usefulness of heteroazophenolic compounds: 3-mercapto-5-(2',4'-dihydroxyphenyloazo-1')--1,2,4-triazol (METRIAP) [18] and 5-(5'-mercapto-1,3,4-tiadiazolo-2'-azo)-2,4--dihydroxybenzoic acid (METIDAREZ- $\beta$ ) [19] for determination of iron in the commonly used pharmaceutic preparations such as: Ferrum - tablets, Ferrum 4 mg + Vit. C effervescens- tablets, Pan Vitan for women - tablets, and Tardyferon<sup>R</sup> – tablets.

For milligram determination of iron (II) quantities in pharmaceutical preparations (Ferrum, Ferrum 4mg +Vit. C, Pan Vitan for women and Tardyferon<sup>R</sup>) 3-mercapto-5-(2',4'-dihydroxyphenyloazo-1')-1,2,4-triazol (ME-TRIAP) [18] and 5-(5'-mercapto-1,3,4-tiadiazolo-2'-azo)-2,4-dihydroxybenzoic acid (METIDAREZ– $\beta$ ) [19] were applied.

### 2. EXPERIMENTAL

Apparatus

- The measurments were carried out with UV-Vis CE 6600 type, Cecil spectrophotometer (CE 6600, Cecil Elegant Technology 6600 Multimode Computing UV Spectrophotometer Nr 86 161, England).
- 3300 type Perkin-Elmer Atomic Absorption Spectrophotometer, Germany;
- PHM type 22 Potentiometer, with a G 2020, glass electrode and K 410 calomel electrode, (Radiometer, Denmark);

## Reagents

- 1. Chemicals of analytical grade from (E. Merc, Darmstad, RFG and POCh Lublin, Poland) were used. Water was deionised.
  - □ Solutions of the dyes of  $c=2 \cdot 10^{-4}$  mol  $1^{-1}$  concentration were obtained by dissolving 0.00547 g of METRIAP and 0.00587 g of METIDAREZ- $\beta$  in

1 ml DMF (dimethyloformamide) and made up with methanol + water (1:1) up to 100 ml.

- □ For measurements the authors used  $FeSO_4$  aqueous solution of  $c=2\cdot 10^{-4}$  mol·1<sup>-1</sup>,
- $\Box$  buffer borate solutions of pH= 7.40 and 10.30,
- $\square$  1 mol<sup>-1</sup> solution of KNO<sub>3</sub>

2. For determination of Fe(II) the authors used the preparations:

- □ Ferrum effervescent tablets produced by N.P. Pharma Company Ltd. on the licence of Dr Schffer Nachf. Gmbh, Germany, series number:708025;
- □ Ferrum 4mg + Vit. C effervescent tablets produced by Pharmavit S.A. Veresegyhaz, Hungary, series number: 0100697;
- Pan-Vitan for women- tablets produced by Bayer Corporation, Elkhart USA, series number: 00250067
- □ Tardyferon<sup>R</sup> tablets produced by Robpharm Ltd Basel, Switzerland, series number: 809718
- 3. METRIAP and METIDAREZ were dissolved in 1 cm<sup>3</sup> of DMF and diluted with methanol + water (1:1)

#### 3. RESULTS

The calibration curves were determined by the described method.

12 ml volumes of the dyes (METRIAP and METIDAREZ $\beta$ ) in methanol of c= 2.10<sup>-4</sup> mol 1<sup>-1</sup> were added to the measuring flasks; aqueous solution of Fe (II) salt stabilized with ascorbic acid (0,4 mg in 100 ml of solution) in the amount from 0.5 to 7 ml as well as 1 mol 1<sup>-1</sup> KNO<sub>3</sub> (in such an amount that the ionic strength was  $\mu$ =0,1) were added to the flasks and made up with a buffer of 7.4 pH (METIDAREZ $\beta$ ) and 10.3 pH (METRIAP).

Absorbance measurements were performed at the analytical wavelength of  $\lambda$  anal = 490 nm (METRIAP) and 600 nm (METIDAREZ $\beta$ ) after 30 minutes in the presence of the reference agent (specially buffered dye solutions with the addition of 1mol 1<sup>-1</sup> KNO<sub>3</sub> in such a quantity that  $\mu$ =0.1). Straight-line course of the calibration curves was noted (in agreement with the Lambert-Beer law) in the range of the following concentrations: 0.05 – 1.45 µg·ml<sup>-1</sup> (METIDAREZ–- $\beta$ ) and 0.05–2.011 µg·ml<sup>-1</sup> (METRIAP). The correlation coefficient (r) was respectively: 0.9980 (METRIAP) and 1.000 (METIDAREZ $\beta$ ); there were also good values of the regression equation (y = ax ± b) ; y = 0.262x – 0.021 (METRIAP) and y = 0.070x + 0.008 (METIDAREZ- $\beta$ ).

Preparation of solutions of Ferrum, Ferrum 4 mg +Vit. C, Pan Vitan for women and Tardyferon<sup>R</sup> for determination of Fe (II).

The tablets of the investigated preparations were mineralised in the folloning way one tablet of the preparation: (Ferrum, Ferrum 4 mg +Vit. C, Pan Vitan for women or Tardyferon<sup>R</sup>) was placed in a conical flask. 10 ml 30%  $H_2O_2$  were added and the tablets were allowed to degrade; next 1 ml dropwise of the concentrated H  $_2$  SO<sub>4</sub> (d = 1.84 g cm<sup>-3</sup>) was added to each flask and all was heated until  $H_2O_2$  was decomposed. Then, 10 ml of 30%  $H_2O_2$  was added again to the flasks and their content was heated. The procedure was repeated three times, next the solutions were evaporated to about 5 ml. 10 ml of water was added and the content was filtered to 100 ml measuring flasks. The conical flasks and the filters were washed four times, filtered and the filtrate was added to the filtrate in the flasks and made up with 100 ml quantitly of water.

#### Preparation of solutions for Fe (II) determination

3 ml sample of Ferrum mineralisate, 10 ml sample of Ferrum 4 mg+ Vit.C mineralisate, 4 ml sample of Pan-Vitan for women mineralisate and 1ml mineralisate of Tardyferon<sup>R</sup> were added each to 150 ml measuring flasks; 0.4 g of ascorbic acid (to reduce Fe (III) to Fe (II) and to avoid oxidation of Fe (II) to Fe (III) ) was added. The flasks were made up with redistilled water up to the line ("solutions for determination").

## Determination of Fe (II) content

12 ml methanol solutions of METRIAP and METIDAREZ- $\beta$  of c= 2·10<sup>-4</sup> mol 1<sup>-1</sup> were added to the measuring flasks. 4 ml (Ferrum), 3 ml (Ferrum 4 mg + Vit.C), 3 ml (Pan-Vitan) and 1 ml (Tardyferon<sup>R</sup>) samples of "solutions for determination" were added. 1 mol 1<sup>-1</sup> KNO<sub>3</sub> (in such a quantity that the ionic strength was  $\mu = 0.1$ ) was added to the flasks and made up with a buffer of 7.4 pH (METIDAREZ- $\beta$ ) and 10.3 pH (METRIAP). Absorbance measurements were made at the analytical wavelength of  $\lambda = 490$  nm (METRIAP) and 600 nm (METIDAREZ- $\beta$ ) after 30 min against the standard solution :12 ml of the dye of c= 2·10<sup>-4</sup> mol 1<sup>-1</sup> with addition of 1mol 1<sup>-1</sup> KNO<sub>3</sub> in such a quantity that  $\mu = 0.1$ , and of a buffer up to 25 ml.

The results of Fe (II) determination of 10 series (n = 10) were elaborated statistically, compared with the Fe (II) determination results obtained with AAS (Perkin-Elmer,  $\lambda$ =248, 3 nm acetylene-air) and presented in Table 1.

	Amount of Fe(II) (mg) declared	Determi- nation of Fe(II) in		Determined of Fe(II) with Metriap (Mp) and METIDAREZ-β (M-β)							
Prepa- ration			The dye	SD			$\overline{x} + t\overline{S}$	Error in relation to the quantity			
	in 1 tablet	by AAS mg			RSD	$\overline{S} = S/\sqrt{n}$	mg	Deter- mined by AAS	Declared		
Ferrum	18 0000		Мр	0.281	1.57	0.089	17.87 ± 0.201	+2.85	-0.74		
	10.0000	17.3750	М-β	0.293	1.63	0.093	17.99 ± 0.21	+3.86	-0.04 -3.47		
Ferrum 4 mg + Vit. C	4 0000		Мр	0.057	1.42	0.018	3.99 ± 0.04	-2.55	-0.06		
	4.0000	4.1020	М-β	0.102	2.55	0.032	4.02 ± 0.07	-0.21	+0.39 +2.55		
Pan- Vitan for women	21,0000		Мр	0.332	1.58	0.105	20.98 ± 0.24	+5.19	-0.34		
	21.0000	19.8950	Μ-β	0.339	1.62	0.107	20.98 ± 0.24	+5.45	-0.11 -5.26		
Tardyfe- ron	80.0000		Мр	1.505	1.87	0.476	80.31 ±1.08	+1.65	+0.39		
		79.0030	Μ-β	0.498	0.62	0.157	80.30 ± 0.36	+1.64	+0.36 -1.25		

Tab.	1.	The	results	of	Fe(II)	determination	with	the	statistical	evaluation	for	10
measurements $(n = 10)$ at 95% probability level												

$$\label{eq:mp_mp} \begin{split} Mp &= METRIAP \\ M{\textbf{-}}\beta &= METIDAREZ{\textbf{-}}\beta \end{split}$$

#### 4. DISCUSSION

Fe (II) ions form chelate bonds of L: Fe = 3:1 composition in watermethanol medium of pH=10.3 (METRIAP) and 7.4 (METIDAREZ  $\beta$ ). The colour of the newly formed complexes under these conditions was red –brown with METRIAP and violet with METIDAREZ  $\beta$ . The reaction of complex formation occurs very quickly, and the created connections were stable for 24 hours. The values of the stability constants expressed as log K were 16.46 with METRIAP [18] and 14.25 with METIDAREZ  $\beta$  [19]. The absorbance coefficient at the analytical wavelength was  $1.42 \cdot 10^{-4}$  at  $\lambda$ =490 nm (METRIAP)<sub>3</sub> Fe(II) and  $0.41 \cdot 10^{-4}$  (METIDAREZ  $\beta$ )<sub>3</sub>Fe(II) at  $\lambda$  = 600nm.

These properties were utilized in the spectrophotometric determination of milligram quantities of Fe (II) in Ferrum, Ferrum 4 mg +Vit. C, Pan Vitan for women and Tardyferon<sup>R</sup>. The precision of the results, expressed as the confidence interval at the 95% of the probability level, was from  $\pm$  0.081 to  $\pm$  1.080 with METRIAP and from  $\pm$  0.044 to  $\pm$  0.263 with METIDAREZ  $\beta$ . The standard deviation in Fe determination with the use of METRIAP was from -0.494 to +0.239 (Ferrum); from -0.108 to 0.083 (Ferrum 4 mg + Vit. C) ; from -0.526 to +0.558 (Pan Vitan for women) and from -2.282 to +1.692

(Tardyferon<sup>R</sup>), and with the use of METIDAREZ  $\beta$ : from -0.666 to +455 (Ferrum); from -0.1080 to +0.1085 (Ferrum 4 mg +Vit C); from 0.3581 to +0.727 (Pan Vitan for women) and from -0.517 to +0.726(Tardyferon<sup>R</sup>).

The percentage error of Fe (II) determination in relation to the declared amount was on average -0.20% (METRIAP) and +0.15% (METIDAREZ  $\beta$ ); while using the AAS method -1.86%, and in relation to AAS method +1.70% (METRIAP) and 2.61% (METIDAREZ  $\beta$ ). Medium percentage values of the variability coefficient were 1.61 (METRIAP) and 1.42 (METIDAREZ  $\beta$ ). Taking into account the relative value of the error made during determination of Fe (II) in multivitamin preparations with microelements (Ferrum, Ferrum 4 mg +Vit. C, Pan Vitan for women and Tardyferon<sup>R</sup>), it can be concluded that the best compound for iron determination is METIDAREZ  $\beta$ . Spectrophotometric determination of Fe (II) carries an error smaller than AAS, as well as taking into account the sensitivity and repeatability of the determination results it appears that both 3-mercapto-5- (2',4'-dihydroxyphenyloazo-1')-1,2,4-triazol (METRIAP) and 5-(5'-mercapto-1,3,4, tiadiazolo-2'-azo)-2,4-dihydroxybenzoic acid (METIDAREZ- $\beta$ ) can be successfully applied for Fe(II) determination in multivitamin pharmaceutical preparations with micro-elements.

#### **5. CONCLUSIONS**

The advantage of the elaborated method of Fe (II) determination in multivitamine pharmaceutical preparations with microelements is great precision and repeatability of the determination results. The determination results imply a smaller error as compared with AAS method. The method is simple and economic and is characterized by a great sensitivity.

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